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Review

Osmoregulation in anthozoan–dinoflagellate symbiosis

Anderson B. Mayfield *, Ruth D. Gates

University of Hawaii, Hawaii Institute of Marine Biology, PO Box 1346, Kaneohe HI 96744, USA

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Abstract

Endosymbiosis creates a unique osmotic circumstance. Hosts are not only responsible for balancing their internal osmolarity with respect to the external environment, but they must also maintain a compatible osmotic environment for their endosymbionts, which may themselves contribute to the net osmolarity of the host cell through molecular fluxes and/or exchange. Cnidarian hosts that harbor intracellular dinoflagellates (zooxanthellae) are excellent examples of such a symbiosis. These associations are characterized by the exchange of osmotically active compounds, but they are temporally stable under normal environmental conditions indicating that these osmotically driven exchanges are effectively and rapidly regulated. Although we have some knowledge about how asymbiotic anthozoans and algae osmoregulate, our understanding of the physiological mechanisms involved in regulating an intact anthozoan–dinoflagellate symbiosis is poor. Large-scale expulsion of endosymbiotic zooxanthellae, or bleaching, is currently considered to be one of the greatest threats to coral reefs worldwide. To date, there has been little consideration of the osmotic scenarios that occur when these symbioses are exposed to the conditions that normally elicit bleaching, such as increased seawater temperatures and UV radiation. Here we review what is known about osmoregulation and osmotic stress in anthozoans and dinoflagellates and discuss the osmotic implications of exposure to environmental stress in these globally distributed and ecologically important symbioses.

Keywords: Bleaching; Coral; Global warming; Glycerol; Organic osmolytes; Osmoregulation; Osmotic stress; Symbiosis

Contents

* Corresponding author. Tel.: +1 808 236 7420; fax: +1 808 236 7443.
E-mail address: mayfield@hawaii.edu (A.B. Mayfield).

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1. Osmoregulation in endosymbiosis: a unique physiological scenario

Anthozoan–dinoflagellate symbioses represent challenging and unique osmoregulatory scenarios. The host contains from one to eight intracellular symbionts of different physiological ages within a specific compartment inside its gastrodermal cells and maintains a dialogue with its dinoflagellate inhabitants that is characterized by an exchange of metabolites (Muscatine and Cernichiari, 1969; Muscatine et al., 1998). As a result, the host must balance its extracellular osmolarity with an intracellular environment that is influenced by both its own metabolism and that of its symbionts. The symbiont's extracellular milieu is defined by the activities of the host cell, that of other symbionts within the host cell, and the host's ability to ameliorate extracellular osmotic pressures (Fig. 1). In this sense, zooxanthellae can be seen as highly osmotically active organelles.

It has been demonstrated that symbiotic zooxanthellae live within an osmotically different environment from that of free-living dinoflagellates (Goiran et al., 1997). However, the processes by which this compatible osmotic environment is established and maintained are not currently understood. While the osmoregulatory components of both bacteria-fish organ symbioses (Dunlap, 1985) and nematode parasites in human digestive tracts (Fusé et al., 1993) have been researched, endosymbiotic zooxanthellae within anthozoan cells have never been examined in this context. Understanding the osmotic relationship between dinoflagellate symbionts and host anthozoans, which include both corals and sea anemones, will help us better understand both the maintenance and the breakdown of these important symbioses.

Although little is known about the osmoregulatory mechanisms employed by symbiotic sea anemones and even less about corals, the maintenance of cell shape is fundamental to life. Thus, it is likely that compounds which play a pivotal role in osmoregulation in other organisms may also function in anthozoan–dinoflagellate associations. For example, photosynthetic dinoflagellates translocate newly fixed carbon to the host primarily in the form of glycerol (Muscatine, 1967), and a component of the host cell environment that triggers this translocation is a suite of amino acids (Gates et al., 1995, 1999). While much of the glycerol translocated to the host is rapidly respired, it is clear that the host maintains temporally dynamic pools of both glycerol and amino acids within its tissues, and these pools decrease in response to temperature-induced shifts in symbiotic metabolism (Gates and Edmunds, 1999). Thus, there is potential for these molecules to function in osmoregulation, especially considering the fact that a compatible osmotic environment within coral cells is necessary for the integrity of the symbiosis (Seibt and Schlichter, 2001). With the goal of understanding how this osmotic equilibrium is achieved through molecular contributions from both the anthozoan host and dinoflagellate symbionts, we will discuss the osmoregulatory strategies utilized by other organisms while introducing the terminology associated with osmoregulation, and then review what is known about mechanisms of osmoregulation in anthozoans and algae.

2. Osmoregulation fundamentals

All cells require a stable environment for optimal metabolic function. To achieve such stability, cells with semipermeable membranes must constantly adjust their cell volume to maintain

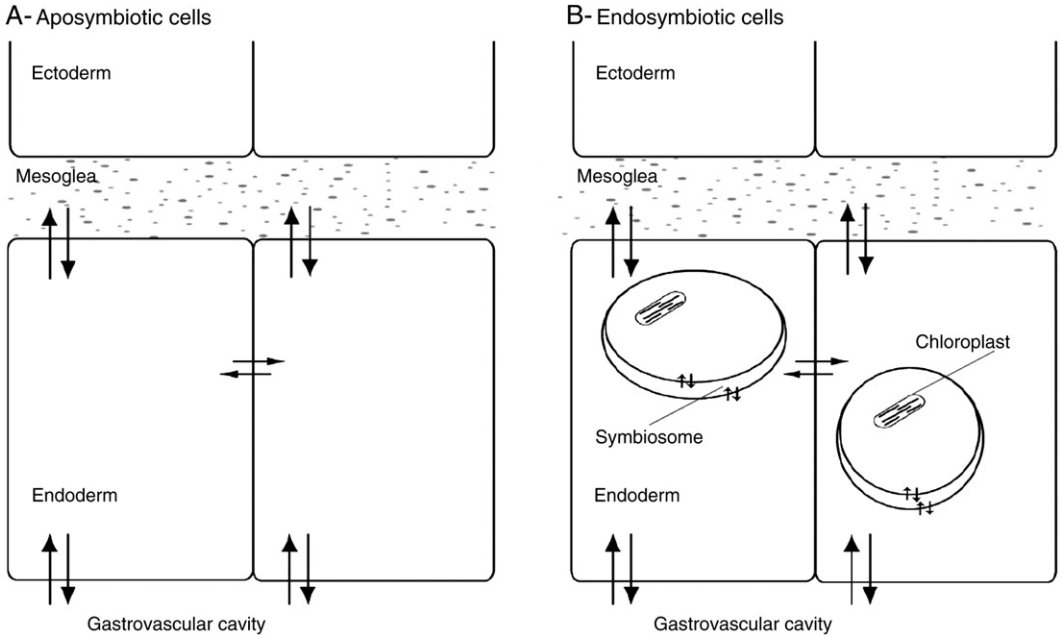

Fig. 1. Symbiotic (A) and aposymbiotic (B) anthozoans cells. Arrows represent flow of osmolytes (free amino acids, glycerol and other polyols, and ions). Cell organelles are assumed to be the same in each condition and have been omitted (i.e., nuclei, mitochondria, etc.).

the equilibrium between intracellular and extracellular osmolarity, a process known as osmoregulation. This phenomenon occurs constantly as cells interact with their extracellular environment, a medium which varies in osmolarity under dynamic conditions. Osmoregulation should be distinguished from osmotic stress, which refers to some degree of osmoregulation surpassing basal levels that can be energetically expensive (Hochachka and Somero, 2002). Osmotic stress can occur because of shifts in the osmolarity of the extracellular environment resulting from desiccation, water and salinity stress, and exposure to concentrated solutions. Consequently, it has the potential to dramatically influence the biological function of the cell (Lang et al., 1998). Whereas a certain degree of osmoregulation exists in healthy cells, osmotic stress occurs only when the cell experiences volume and osmolyte fluctuations that compromise macromolecular structure and metabolic function.

Marine organisms are commonly placed into two categories when describing their strategies for living within osmotically dynamic environments. Most marine vertebrates are osmoregulators, or organisms that maintain constant concentrations of osmotically active molecules (osmolytes) within cells regardless of external osmolarity. In contrast, the majority of marine invertebrates are osmoconformers and maintain intracellular osmolyte concentrations equivalent to that of the surrounding medium, seawater. While the two approaches may seem fundamentally different, the intracellular osmolarity is extremely dynamic in both osmoregulators and osmoconformers, with metabolic activities constantly producing and depleting osmotically active substances as changes in intracellular and extracellular osmolarity are detected (Timasheff, 1992). The ability of cells to measure changes in osmotic concentration through osmosensors, structures that detect fluctuations in water pressure or ion concentrations, has been well studied in model organisms such as yeast (Brewster et al., 1993) but is virtually unexplored in anthozoans.

3. Mechanisms of osmoregulation in anthozoans

Labeling corals and sea anemones, as well as many other marine invertebrates as “osmoconformers” diminishes the fact that these organisms dedicate a large amount of their energy to regulating their cellular volumes and solute concentrations (Somero and Yancey, 1997). Anthozoans, like other invertebrates, tend to disfavor altering cell volume and intracellular ion concentrations when equilibrating with external osmolarity. High and variable concentrations of inorganic ions can cause cellular dysfunction due to the effect on macromolecular structure (von Hippel and Schleich, 1969; Leberman and Soper, 1995), especially those commonly found in seawater such as Na^+, K^+, and Cl^-. Additionally, the uptake of too much water in response to osmotic stress can have more obvious negative impacts such as cytoskeletal and membrane damage. Cells require ideal amounts of both water and osmolytes in order for the myriad of biochemical reactions to take place in solution at the appropriate rates (Atkinson, 1969).

Despite being detrimental to cell function, diffusion and osmosis are inevitable responses in all cells exposed to external osmolarity changes, as these processes occur rapidly. Therefore, it is not surprising that under extreme salinity changes (outside typical daily and seasonal fluctuations) corals, and likely other symbiotic anthozoans, tend to expel symbionts (i.e., bleach; Kerswell and Jones, 2003) or even die (Edmundson, 1928; Coles and Jokiel, 1978, 1992; Hoegh-Guldberg and Smith, 1989) due to their inability to initiate an osmotic stress response before significant changes in cell volume occur. From studies observing such extreme salinity changes, it is perhaps understandable that at one point anthozoans were considered to be stenohaline, and so unable to survive substantial fluctuations in external osmolarity.

Once researchers have began studying effects of environmentally realistic salinities, it was proven that some species are in fact euryhaline and can withstand significant changes in external osmolarity (Coles, 1992; Manzello and Lirman, 2003). For instance, Muthiga and Szmant (1987) found that neither respiratory nor photosynthetic rates of *Siderastrea siderea*, a coral, were affected by changes in salinity of less than 10 psu above or below the acclimation salinity, and no bleaching or mortality was observed. *S. siderea* is known to inhabit environments with fluctuating salinity in South Florida where this project was conducted, so the result was not unexpected. A possible reason for the observed absence of any deleterious effects during the changes in salinity is likely due to the fact that under less extreme extracellular osmolarity changes, a cell-mediated response occurs within several minutes to hours in order to reduce volume and ionic fluctuations and restore cell homeostasis. This is typically achieved through the production of compatible organic osmolytes.

Compatible organic osmolytes (COOs) are molecules synthesized by most marine invertebrate cells that fluctuate in response to osmotic stress and do not disrupt cellular function. COOs are typically either polyols, free amino acids, methylammonium and methlysulfonium solutes, or urea and are synthesized or degraded in order to alter intracellular osmolarity. The most common COOs found in anthozoans based on sea anemone studies appear to be glycerol, a polyol, and free amino acids (Deaton and Hoffmann, 1988; Shick, 1991). COOs are ubiquitous in all forms of life because they can accumulate inside cells without greatly affecting protein structure and function. Likewise, depletion of COOs has minimal impact on cells, whereas substantial water and ion loss can lead to apoptosis (Kültz, 2005).

In addition to their role in cell volume regulation, COOs protect various elements of cells, such as macromolecules and membranes, from the effects of heat stress and desiccation (Back et al., 1979). It has been hypothesized that cells tend to utilize end products of metabolism or other molecules readily available in the cytoplasm as COOs so that less energy is expended during osmoregulation (Bowlus and Somero, 1979). For instance, it would not make sense for cells to produce larger, more complex molecules, such as proteins, in response to an osmolarity change, when simpler molecules or molecules that could be catabolized from larger ones (such as amino acids) to serve as osmolytes would be less energetically costly. As will become clear, symbiotic anthozoans and their dinoflagellate zooxanthellae likely utilize this strategy.

Before discussing osmoregulation in both host anthozoans and zooxanthellae and in the holobiont, the aposymbiotic condition should be discussed. In asymbiotic or aposymbiotic anthozoans the osmotic scenario is less complex, as there is only one contributor to the osmolyte concentration, the anthozoan cell itself (Fig. 1A). In sea anemones lacking symbionts, altering concentration of free amino acids (FAAs) is the primary means of regulating intracellular osmolarity (Shick, 1991; Roberts et al., 2001). Specifically, taurine can account for up to 85% of the free amino acid pool (Deaton and Hoffmann, 1988), though glycine and smaller amino acids are also important. This observation supports older work by Bowlus and Somero (1979) who demonstrated that taurine and glycine in particular do not influence the K_m of essential enzymes such as phosphoenol pyruvate (PEP), whereas inorganic ions have a substantial impact. On the other hand, the evidence strongly suggests that zooxanthellate anthozoans (Fig. 1B) will be relying on both FAAs and glycerol for osmoregulation, especially given glycerol's pivotal role in the symbiosis (Muscatine, 1967; Gates et al., 1995; Gates and Edmunds, 1999).

3.1. Hyperosmotic stress in anthozoans and dinoflagellates

Upon exposure to hyperosmotic conditions, anthozoans and their zooxanthellae will either absorb ions from the environment, release water (the initial, detrimental responses), synthesize COOs directly, or establish pools of COOs by breaking down macromolecules. For instance, one protein has the same value as an osmolyte as a Na^+ ion despite the fact that the protein is far greater in size. However, a protein can be made up of hundreds of amino acids, and each amino acid can function as an osmolyte, so many organisms, and likely anthozoans, can break down large proteins into constituent amino acids to establish a greater intracellular osmolyte concentration if necessary.

As described above, marine invertebrates often maintain intracellular pools of free amino acids in the cell cytoplasm in order to quickly elicit an osmotic stress response. Corals, and likely other anthozoans, maintain FAA pools within cells, and it is thought that these FAAs may additionally serve as a host factor to stimulate photosynthate release from zooxanthellae (Gates et al., 1995). Thus, anthozoans and other marine invertebrates may not need to break down proteins in response to hyperosmotic stress, as they already have FAA pools that can serve as COOs. On the other hand, if amino acids are readily available in the external environment, cells will preferentially incorporate these extracellular osmolytes rather than synthesize new ones, as utilizing these "osmoprotectants" will be energetically favored (Hochachka and Somero, 2002). Zooxanthellae likely do not rely as heavily on FAAs, as many are nitrogen limited and cannot metabolically afford rapid shifts in amino acid levels. That being said, during osmotic equilibrium, zooxanthellae are known to leak FAAs into coral cytoplasm (Fitzgerald and Szmant, 1997; Swanson and Hoegh-Guldberg, 1998).

In addition to FAAs, glycerol, another commonly utilized COO, may also be accumulated under hyperosmotic conditions in corals and other anthozoans. Glycerol, the main carbon source translocated from zooxanthellae into host cytoplasm (Muscatine, 1967), is concentrated in intracellular pools, and it has been shown that the glycerol and FAA pools, which are regulated by both host and symbionts, may be functioning as COOs (Gates et al., 1995, 1999). As it turns out, unicellular algae, and thus potentially dinoflagellate zooxanthellae, break down large starch molecules into polyols such as glycerol (Blackwell and Gilmour, 1991) in response to hyperosmotic stress, and so glycerol may not only be important in maintaining osmotic homeostasis within host cell environments, but also within the zooxanthellae cells themselves.

3.2. Hypoosmotic stress in anthozoans and dinoflagellates

Under hypoosmotic stress, when external osmolarity has decreased, cells need to reduce concentration of osmolytes before water uptake and ion loss occur. This can be done by metabolizing COOs, compartmentalizing them, or excreting them from the cell. Many marine invertebrates, including sea anemones, reduce intracellular osmolyte concentration by decreasing concentration of FAAs. A common means of achieving this is decreasing permeability to FAAs so that they no longer enter the cell from the extracellular environment. Anemones also tend to secrete mucus in response to hypoosmotic stress (Bursey and Harmer, 1979), possibly in order to reduce osmotic influx of water. It has never been demonstrated which of these strategies is utilized by corals, as much of what we know about anthozoan osmoregulation comes from studies on sea anemones (for review, Shick, 1991). Likewise, nothing is known about hypoosmotic response in endosymbiotic zooxanthellae, but *Dunaliella* sp., a, unicellular green algae, appears to achieve homeostasis through decreasing glycerol concentration (Marengo et al., 1985; Chitlaru and Pick, 1991). Thus, zooxanthellae may be reducing glycerol concentration in response to hypoosmotic stress. *Dunaliella* sp. is, however, phylogenetically quite distant from the zooxanthellae, and so assuming that the two taxa function identically under hypoosmotic conditions may be inappropriate. Despite the dearth of information on osmoregulatory mechanisms in corals and their symbionts, there have been several studies looking at the effects of hypoosmotic stress on coral–algal physiology.

Moberg et al. (1997) found that *Porites lutea* and *Pocillopora damicornis* experienced reduced Pg:R (photosynthesis: respiration) ratios upon a salinity decrease from 30 psu to 20 psu, indicating that either the zooxanthellae were decreasing photosynthetic rates or that coral and zooxanthellae respiration rates were increasing relative to photosynthetic yield. The latter scenario could be possible, as increased respiration rates are frequently observed in organisms exposed to increased or decreased salinities (Vernberg and Vernberg, 1972). These researchers did not witness expulsion of zooxanthellae. However, the majority of experiments examining hypoosmotic effects on coral–algal symbioses did observe bleaching (e.g., Marcus and Thorhaug, 1981; Engebretson and Martin, 1994 [anemones]; Titlyanov et al., 2000; Kerswell and Jones, 2003). For instance, large storms and hurricanes that greatly reduce salinity have been shown to elicit zooxanthellae loss (Goreau,

1964; Egana and DiSalvo, 1982). The mechanisms behind low salinity bleaching are unresolved, although Van-Woesik et al. (1995) reported that swelling and rupture of host cells led to low salinity bleaching. Similarly, it has been observed that host cells still containing symbiotic algae have been released from low salinity-shocked corals (Titlyanov et al., 2000). These two studies in particular indicate that corals are unable to regulate their cell volumes under stressful conditions. Thus, while corals and their symbionts almost surely possess means of countering hypoosmotic stress, there is a certain threshold beyond which harmful physiological effects such as symbiont loss or even death are experienced.

4. Osmotic stress in coral–algal symbioses

We have discussed potential mechanisms for countering both hyper- and hypoosmotic stress in anthozoans and their zooxanthellae symbionts, mostly based on sea anemone studies. From this point forth, the discussion will focus on coral–algal symbioses in particular, as they are more critical on a global scale in terms of reef production. Looking at either organism in a mutualistic symbiosis in isolation is inappropriate when considering intracellular osmoregulation. So far, we have discussed the role of glycerol and FAAs as two potential COOs that are utilized by both anthozoans and symbiotic endosymbionts. Glycerol is likely more important as an osmolyte in zooxanthellae since marine algae are typically too nitrogen-limited to rely on rapid changes in amino acid levels. FAAs and glycerol both have primary roles in symbiotic function aside from their hypothesized function as COOs. FAAs compose at least part of the suite of chemicals necessary for the release of zooxanthellae photosynthate into coral cytoplasm (Gates et al., 1995). Also, FAAs are readily leaked from symbionts into host cytoplasm (Fitzgerald and Szmant, 1997). Glycerol is the major source of photosynthate released by zooxanthellae and much of it is rapidly respired by host coral cells (Muscatine, 1967). However, some of the glycerol, in addition to the secreted free amino acids, is maintained in cellular pools (Gates and Edmunds, 1999).

The fact that the symbiosis maintains temporally dynamic and environmentally sensitive pools of these substances strongly suggests a role in osmoregulation. By discussing which events are known to occur during osmotic stress in other systems and combining that knowledge with what we know about coral–algal metabolism, we can determine what will likely occur when a symbiotic coral undergoes osmotic

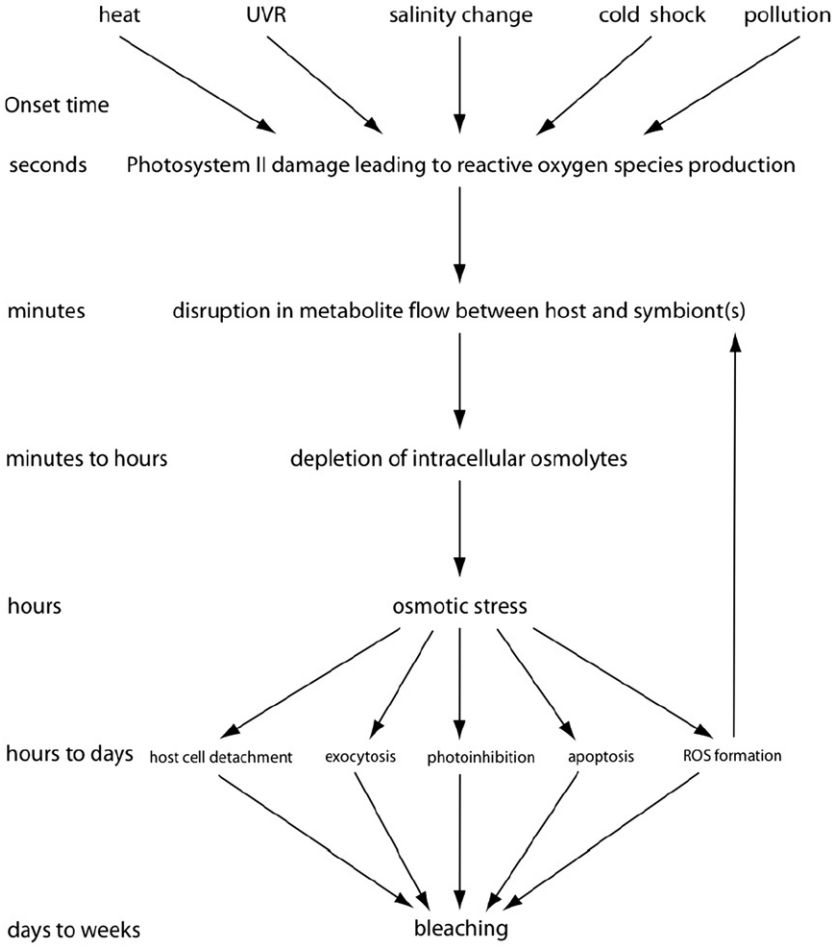

Fig. 2. A flow-chart describing how multiple stressors could elicit coral bleaching as a result of an osmotic stress response. The time of onset of each proposed event is shown on the left.

stress stemming from an environmental perturbation such as temperature change or increased UV radiation. Concurrently, we speculate on the timeframe of the coral cell's response to osmotic stress.

Osmoregulation under steady-state conditions is a constant cellular activity that is exquisitely fine-tuned and relies on multiple layers of biological connectivity. However, any condition that compromises, damages, or inhibits a component of this biological cascade will negatively impact the cell's ability to transport ions across membranes, and/or accumulate compatible osmolytes and will ultimately manifest as increases or decreases in cell volume that exceed the regulatory thresholds (Hochachka and Somero, 1984). The magnitude of these cell volume changes will be dictated by the duration and severity of the disturbance, and this will be reflected in the scope and complexity of metabolic responses in the cell. These include disruption to the cytoskeleton (Chowdhury et al., 1992), cell adhesion dysfunction (Melchior and Steim, 1976), shifts in cytosolic pH, ionic imbalances (Hohmann, 1997), increased respiration (Vernberg and Vernberg, 1972), increased RNA and DNA synthesis (Kültz, 2000), up-regulation of heat shock proteins (Petronini et al., 1993), formation of reactive oxygen species (ROS) and, in the worse case scenario, the initiation of cell death pathways (Kültz, 2005).

Interestingly, almost all of the aforementioned cellular impacts have been documented in cases where corals lose zooxanthellae or their associated pigments, a phenomenon known as bleaching due to the paling of the coral. In fact, the variety of stressors that elicit a bleaching response, and the multiple cellular mechanisms that are responsible for the loss of symbionts from bleaching corals fit well within a biological framework that considers osmoregulation. For instance, high levels of ROS have been found in coral cells undergoing bleaching (Lesser, 1996, 1997; Downs et al., 2002). The ROS originating from the algae may form in direct response to heat and UV stress or after degradation of photosynthetic pathways, especially those involving photosystem II (Warner et al., 1996, 1999; Jones et al., 1998, 2000). This photoinhibition, another proposed factor proceeding bleaching events (Hoegh-Guldberg, 1999), may be a result of an osmotic stress impact on the ability to transfer ions across membranes (Hochachka and Somero, 2002). In photosystem II, the pumping of H^+ ions into the thylakoid and the conversion of ADP+P into ATP are driven by electron gradients established in the thylakoid membrane. Because ion flow across membranes could be disrupted during osmotic stress, essential steps of photosynthesis may not occur, and consequent photosystem breakdown could lead to generation of free oxygen radicals. The ROS may also stem simply from increased metabolism in response to the osmotic stress response, which involves catabolism or anabolism of COOs and formation of heat shock proteins depending on the extent of protein denaturation from cell volume and ion changes (Cohen et al., 1991).

We feel that our understanding of coral bleaching may be significantly improved by defining the osmoregulatory mechanisms in coral–dinoflagellate symbioses, and by clarifying their role, if any, in mediating the cellular events that ultimately culminate in coral bleaching. We do not know the exact mechanism for the onset of osmotic stress within coral cells housing endosymbionts, but based on what is known from the few studies on coral osmoregulation and the larger body of literature on coral–algal metabolics, we can hypothesize one particular scenario, described below, that would involve osmotic stress in a bleaching response (Fig. 2).

Let us consider the situation of a zooxanthellate coral experiencing conditions that would normally elicit a bleaching response such as increased temperature or UV radiation. Such conditions can lead to photosystem II damage in the symbionts, which results in production of ROS (Lesser, 1996). This initial response occurs quite rapidly, and ROS can form after only several seconds of the algae having lost their ability to dissipate light energy (Richier et al., 2005, 2006). Photosynthesis is impaired (Jones et al., 1998), and translocation of photosynthate from algae to host cytoplasm is reduced, resulting in a depletion of glycerol and, to a lesser extent, free amino acids in the coral cell's intracellular pools (Gates and Edmunds, 1999). This will cause water to begin exiting the cell (osmosis) with potentially perturbing ions entering via diffusion. This hyperosmotic stress response could occur as soon as only several minutes after the halt in glycerol flow from symbionts into host cytoplasm. The coral's proteins will begin to denature as cell pH and voltage change from the water loss and charge shift. Heat shock proteins are produced to refold denatured proteins or prevent unfolded ones from aggregating (Downs et al., 2000; Brown et al., 2002a).

As cell volume changes due to water loss, cytoskeletal elements begin to fracture (Fang et al., 1998), and cell adhesion proteins may detach, leading entire cells to separate from the organism (Gates et al., 1992). For cells still intact and attached, compatible organic osmolytes like glycerol are rapidly produced to increase the intracellular osmotic concentration. Free radicals produced from molecular degradation of proteins and the consequent increase in metabolism (Lesser, 1996, 1997; Halliwell and Gutteridge, 1999) from the osmotic stress interact with macromolecules, causing substantial cellular damage. This ROS production could serve to exacerbate the harmful oxygen species effect initially stemming from photosystem II breakdown at the onset of the algal stress response, which would then further contribute to the disruption of metabolite flow from symbiont to host, leading to a feedback loop of multiple stresses (Fig. 2). This detrimental feedback loop could occur as soon as several hours after the onset of the ultraviolet, temperature or other stressor that elicited the initial oxidative stress and consequent disruption of metabolite flow.

At this point, the algae, which are likely experiencing rapidly varying glycerol levels, as well, and can typically survive outside of hosts, may exit the cell. Likewise, the coral cell, which is expending more energy on mitigating a problem stemming partially from its symbiont, may decide that it is better off without it/them. The breakdown could occur in as little as a few hours if the stress was significant enough, but would more likely occur after several days of exposure to the stressor, when the holobiont has exhausted its energy reserves needed to restore homeostasis in the osmotically disrupted cell.

If the holobiont remains intact under such stressful conditions, exocytotic or apoptotic events could ensue, as well (Fig. 2). This is speculative, but we have reason to believe that osmotic stress, or, in general, failure of coral and algae to maintain a compatible osmotic environment, could lead to the breakdown of the symbiosis.

There has been at least one study demonstrating that a compatible osmotic environment within coral cells is necessary for the integrity of the symbiosis (Seibt and Schlichter, 2001). In this work, the authors looked at varying intracellular ionic composition, as opposed to compatible organic osmolytes, and found that particular levels maintained by the coral cells improve carbon assimilation by zooxanthellae. Thus, the chemical dialogue between partners is promoted under ideal osmotic conditions. We are interested in observing the other side of the coin: what happens when heat, UV, or any other bleaching-inducing stress causes a disruption of the osmotic equilibrium established in these cells? Our current understanding of the biochemical interactions between host and symbionts is not perfect and requires further study (Edmunds and Gates, 2003), but we know enough about the flux and concentrations of glycerol and amino acids within coral cells to realize their potential to serve as compatible organic osmolytes necessary for symbiotic integrity and cellular homeostasis for both host and endosymbionts.

5. Further research

One way to proceed in elucidating the involvement of an osmotic disturbance in the bleaching response is to determine whether conserved elements of osmosensory pathways found in other organisms exist in corals. Osmosensory pathways in yeasts and basal metazoans are generally made up of two components, the osmosensors and the response regulators. When the osmosensors are inactive, they phosphorylate the response regulators, which repress the downstream elements in the pathway. During stressful conditions, the osmosensors are activated, they no longer phosphorylate the response regulator, and the response regulators activate downstream intermediates, which ultimately induce the expression of transcription factors required for synthesis of glycerol or other COOs (Kültz and Burg, 1998).

A genetic analysis of these pathways reveals that the amino acid sequences for two components, HOG1 and GPD1 (yeast gene names), are highly conserved across phylogenetically distant taxa (Bohm et al., 2002). HOG1 (high osmolality glycerol) is an intermediate located downstream of the response regulator and is a member of the highly conserved mitogen-activated protein kinase (MAPK) family (Fig. 3), a group of genes that have been demonstrated to function in an osmosensory capacity in all eukaryotes studied to date (Winkler et al., 2002; Cowan and Storey, 2003; Mao et al., 2004). GPD1 (glycerol 3-phosphate dehydrogenase), whose activation is controlled by the HOG pathway, is required for the reduction of dihydroxyacetone phosphate, the primary substrate for glycerol formation, and is also an important protein involved in the osmotic response (Remize et al., 2003).

MAPK cascades exist in some unicellular algae but do not function as part of osmoregulatory cascades (Lin and Zhang, 2003). Thus, zooxanthellae likely do not utilize such pathways for osmoregulation. Whether or not similar pathways exist in corals is unknown. If such MAPK cascades are found in corals, then we can use molecular techniques to quantify transcription of genes involved in the osmotic stress response during bleaching events to see if their rates are greater than during more stable conditions. In the hypothetical scenario above in which a hyperosmotic stress is occurring, we would expect initiation of glycerol producing pathways. Such information will tell us whether or not the glycerol pools are being used in osmoregulation. Up-regulation of these genes could occur after only several minutes of stress exposure, meaning that daily sampling, as is common in many coral health studies, may be inappropriate for elucidating important molecules of the holobiont's stress response.

6. Coral bleaching theories and considerations

So far we have mentioned the phenomenon of coral bleaching as a secondary stress response to other stresses, namely osmotic stress. Coral bleaching is largely considered to be one of the greatest threats to the world's coral reefs (Wilkinson, 1999; Pandolfi et al., 2003). Consequently, over the past 15 years, an increasing amount of research has focused on attempting to elucidate a mechanism for this phenomenon (e.g., Gates et al., 1992; Brown, 1997; Hoegh-Guldberg, 1999). In other words, there is great interest in discovering *how* corals bleach. Likewise, a great deal of research

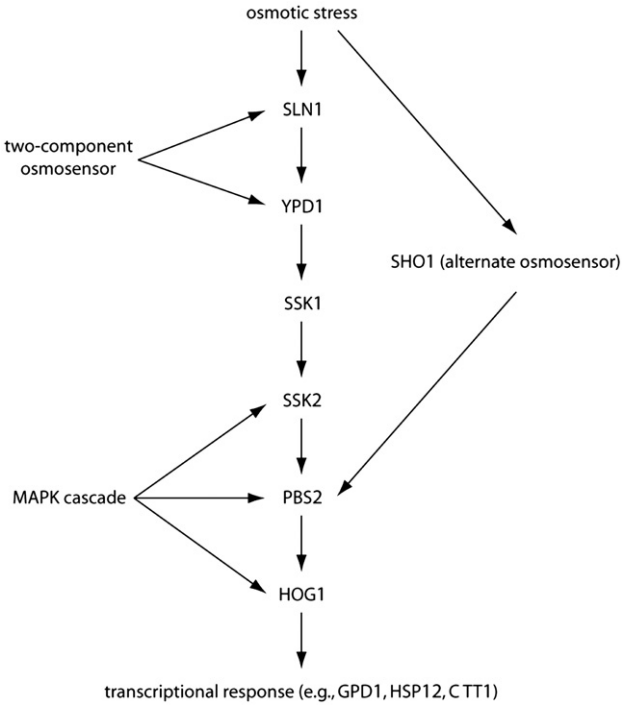

Fig. 3. An overview of osmosensory pathways in yeast. Osmotic stress activates the osmosensors SLN1 and SHO1, which ultimately activate the high osmolality glycerol (HOG1) protein. HOG1 initiates production of glycerol through regulation of transcription of proteins involved in glycerol production, such as GPD1.

has gone into discovering which environmental stressors *cause* corals to lose their symbionts (Gates, 1990; Glynn, 1991, 1993; Gleason and Wellington, 1993). In this context, exposure to higher temperatures and increased levels of UV radiation have received the bulk of the research attention, but other stressors such as salinity changes (Fang et al., 1995; Van-Woesik et al., 1995), cold stress (Muscatine et al., 1991), and bacterial infection (Kushmaro et al., 1996) have all been demonstrated to elicit bleaching as well. In short, nearly any environmental disturbance can result in a bleaching response.

We propose that any environmental change that alters the metabolite exchanges between host and symbionts (and so alters osmolyte pool levels), could potentially lead to an osmotic stress response and elicit and account for many if not all the mechanistic observations on bleaching corals, such as ROS formation, protein damage, and photoinhibition, the latter of which being potentially already damaged directly from heat or UV stress. The goal of this review has been to promote thinking about this endosymbiotic system differently by demonstrating that an osmotic stress response can reconcile all of the biochemical and histological observations that have been made to date and used as rationale for proposing the mechanisms by which corals bleach.

7. Conclusions

Corals and their endosymbionts maintain pools of small, organic molecules within the coral cells (Gates and Edmunds, 1999). Rapid fluctuations in these biochemical pools due to environmental stress may lead to osmotic stress and ultimately the degradation of the symbiosis. Understanding how corals osmoregulate and acclimate at the biochemical level to changing environmental conditions (Buddemeier and Fautin, 1993) is important in predicting whether or not coral reefs will persist in the face of climate change and other anthropogenic disturbances (Knowlton, 2001; Coles and Brown, 2003), and thus sheds light on both coral resistance to stress (Brown et al., 2002b; West and Salm, 2003) and resilience to episodes of chronic perturbation (Lang et al., 1992; Connell, 1997; Obura, 2005).

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